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TITLE: Regeneration of Food Flavors Through Enzymatic Action

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ABSTRACT: The discovery was made that the natural flavor of fresh foods can be regenerated in processed foods through the catalytical action of specific enzymes on specific chemicals called "flavor precursors." These, being more stable than the enzymes, survive, at least in part, the food processing operations. The new process applies to a variety of canned, frozen, dehydrated, or irradiated foods.

Flavor enzymes may be obtained not only from fresh foods, but also from a variety of sources and even from waste products. It was also found that the flavor enzymes may be obtained from non-food sources, as for example, fungi.

The "flavor precursors" belong to a variety of chemical systems. Most thoroughly investigated are the thioglucosides, the "flavor precursors" of the Cruciferae, or the cabbage family. A total synthesis of Cruciferae thioglucosides was developed. It was found that synthetic thioglucosides are just as effectively attacked by specific enzyme systems as the natural ones.

The process as applied to white cabbage will be described in detail.

SESSION C-I-4

TITLE: Some Problems Associated with the Development of a Process for a Biological Agent

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ABSTRACT: Problems associated with the development of a process for the manufacture of a biological agent are described. The type of biological agent and the assay technique required to detect its presence are reported. The scope of the project is related to the processing problems experienced at the pilot plant level. Processes are described, including the form and activity of the final product.

REGENERATION OF FOOD FLAVORS THROUGH ENZYMATIC ACTION

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The problem of restoring to processed foods the aroma characteristics associated with the fresh product is one which is well recognized by anyone who has been subjected to Army rations. It is generally recognized that the major factor in the acceptance of food is based on the sensory perception of odor, taste and texture. Of these, we are most sensitive to odor.

The amount of compounds giving the characteristic odors associated with the fresh fruits and vegetables is extremely small, represented in many cases by parts per million. Thus the study of the original food flavors, and the problem of restoring flavor are of considerable difficulty. One of the factors that have made this particular type of work successful has been the recent application of new, extremely sensitive means of analytical detection of volatile compounds (1, 2). The small quantities of volatile substances, which we associate with aroma, are related to less volatile compounds which we call flavor precursors. These are more stable and less likely to be lost or destroyed during processing treatments. The bulk of the food gives texture or body and the fundamental food nutrients such as protein, carbohydrates, fats, etc.

When a food is processed we can distinguish between the original fresh flavor and an altered flavor, which may or may not be desirable. In many cases the processed flavor has now become accepted and is characteristic of the processed food without relating it to the fresh flavor. As an example, canned peas have a flavor which is entirely different from that of the fresh product, yet most people find the canned product completely acceptable. It is, in fact, a different product. An off-flavor is an aroma we do not associate with a certain food. Yet it may normally occur in other foods. For example, acetaldehyde is a normal constituent of most fruit aromas, but it is definitely an off-flavor in butter where it sometimes results from microbial action. Hydrogen sulfide and dimethyl sulfide are important

components of beer flavor where they are present in amounts below the threshold of olfactory sensitivity. In other foods they might be evidence of putrefaction.

The aroma components of foods are usually associated with the following classes of organic compounds: aliphatic acids, esters, aldehydes, ketones and alcohols. In addition, minute quantities of nitrogen- or sulfur-containing chemicals and terpenes may be present.

The problem with which we are concerned is how can we restore to a processed food all or part of the aroma associated with fresh food. In the first slide

SLIDE 1

is shown schematically the concept of flavor regeneration which we have developed. The first block shows the presence in the fresh product of a considerable amount of natural flavor along with latent flavor precursors and active enzymes. Through processing treatments, these volatile natural flavors are largely lost and in part are replaced by altered flavor as shown in the second block. The flavor precursors are more stable and survive the processing treatment. The activity of the enzymes is lost by the heat treatment involved. If we can demonstrate that certain enzymes will now react with the flavor precursors, it should be possible to restore some of the natural flavor to the processed food. This has been done and is shown in the third block.

One could very quickly ask why process foods if this is going to result in the loss of the original fresh flavor. We believe the answer is obvious. The processing of food is basically a stabilizing process. The stabilization is achieved by substantially destroying or inhibiting microorganisms and inactivating enzymes, either through freezing, heat-sterilization, the use of ionizing radiation, dehydration, or simply by drying, the latter being the method handed down to us from prehistoric times. These stabilizing processes prevent or retard chemical changes, and deterioration through bacterial action. Thus while we achieve the goal of stabilizing food, we have done so at the price of freshness.

In this study we have made the basic observation that potential flavor, in the form of flavor precursors, may be converted into fresh flavor through the action of the proper enzyme added at the time of reconstitution of the food, or in its preparation for consumption (3, 4). In order to obtain the maximum flavor regain, it is necessary to understand the nature of the enzyme precursor reaction. Where a dehydrated product is used, water is required, and the reaction must proceed at the proper pH and temperature. The next slide

SLIDE 2

demonstrates the reconstitution of flavor in dehydrated cabbage. The gas chromatograph of head space above freshly minced cabbage shows the presence of allyl isothiocyanate and butyl isothiocyanate. These are important aroma constituents of the fresh product. The identity of these compounds has been established by gas chromatography and mass spectrometry. In the case of the dehydrated cabbage, these compounds are absent. The sensitivity of the method is such that the absence of a peak is also evidence for absence of detectable odor. Now when an enzyme preparation is added to the dehydrated cabbage, the two isothiocyanates reappear (1). This provides analytical evidence for restoration of two important flavor compounds. The next slide

SLIDE 3

shows other isothiocyanates and sulfides which have been found in cabbage, (1, 5, 6) through analysis by gas chromatography, mass spectrometry and by conventional isolation and characterization methods. It may be of interest to note that in securing the data shown in this slide approximately 100 grams of cabbage were required while by contrast our earlier studies required 100 lbs of cabbage in order to extract and separate out the flavor constituents.

The enzymes used for the reconstitution of flavor may be isolated from the fresh food, or from one of its waste products such as leaves, stems, etc. In the establishment of a commercially successful system for reconstituting flavor, an economical source of enzymes must be obtained. As part of this study, we have found that the fungus, *Aspergillus sydowi*, is capable of producing the required enzyme (a thioglucosidase), and that the yields of enzyme are much better than those obtained from higher plants. This fungal enzyme has been shown to be effective in the release of isothiocyanates from the thioglucoside flavor precursors (7).

While the basic principle is established that enzymes are involved in the release of certain flavor components, it is by no means certain that all flavor components arise in this fashion. As shown in this slide (slide 3) we have observed 20 volatile sulfur compounds in fresh cabbage (1). With the exception of the isothiocyanates, and possibly certain sulfides, we have yet to show the enzymatic origin of the other components. We are confident, however, that the isothiocyanates play the major role in the fresh aroma characteristic of cabbage and other members of that family because of their pungent odor. It is known that there are about 25 naturally occurring isothiocyanates (mustard oils). The precursors of these isothiocyanates is the family of compounds called thioglucosides. The *in vitro* enzymatic hydrolysis of these compounds has been studied in detail by Ettlinger et al. (8) who have made the interesting observation that Vitamin C acts as a co-enzyme. The reactions are shown in the next slide

SLIDE 4

The addition of the vitamin increases the release of the isothiocyanate. This slide shows the typical equations for thioglucosides undergoing enzymatic hydrolysis, through two intermediates, to form isothiocyanates and nitriles. The ascorbate-activated enzyme cleaves the β-glucoside (I) to form the unstable thiohydroxamate ion (II) which is spectrophotometrically detectable and in turn undergoes Lossen rearrangement at neutral pH to form isothiocyanates and sulfate. At more acid pH, nitrile and sulfur appear as decomposition products of the thiohydroxamic acid (III).

The isothiocyanates vary in their aroma characteristics. Some are pungent as for example the allyl isothiocyanate, while others are mildly pleasant such as the methyl-thiomethylisothiocyanate (9). The subtle characterization of any food aroma is probably due not to any single chemical entity but rather to a mixture of volatile compounds.

As part of this work a general synthesis of thioglucosides (10) permitting the synthesis of new compounds has been achieved as shown in the next slide

SLIDE 5

In what might be regarded as the simplest possible scheme for synthesizing thioglucosides (I), an aldoxime can be chlorinated and without isolation the resulting hydroxamyl chloride sulfonated with sulfur trioxide in dimethylformamide to a hydroxamyl chloride-O-sulfonate, V, isolated as a potassium salt. Condensation of the chloride with sodium glucopyranosyl mercaptide in aqueous solution formed the thioglucoside (I) anion isolated by ion exchange conversion to a crystallizable salt.

Another flavor enzyme system in cabbage is that which converts the precursor, a sulfoxide, to the volatile flavor constituent disulfides. These sulfides along with the isothiocyanates represent the important characteristics of the aroma of cabbage. The enzymatic- and acid-hydrolyses of S-methyl-cysteine sulfoxides (2, 11, 12) are shown in the next slide

SLIDE 6

Starting with the sulfoxides intermediates are shown to occur prior to the formation of the dimethyldisulfides.

In the last slide

SLIDE 7

are shown other processed foods which have been similarly treated with enzymes, and which have been shown by sensory evaluation to have

enhanced flavor regeneration (4). From this table it can be seen that a large number of common foods may have flavor regeneration systems comparable to those in cabbage. It is now necessary to establish the nature of the enzymes in these foods in which the release of flavor has been shown organoleptically, and to determine the nature of the flavor precursors involved.

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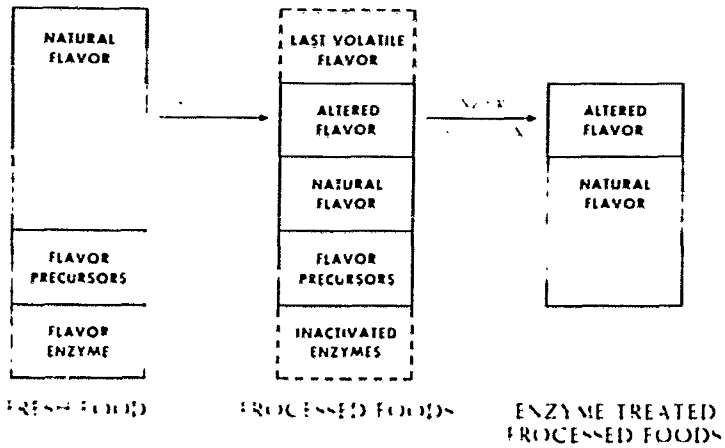
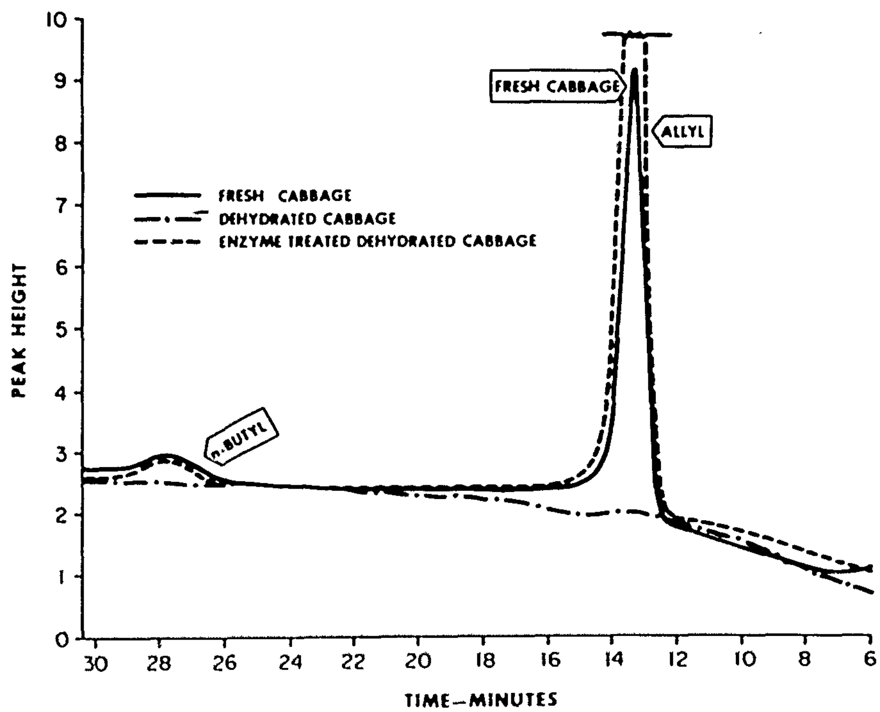


Slide 1. Regeneration of Natural Flavor



Slide 2. Flavor Release by Enzymes

ISOTHIOCYANATES
- Methyl
- n-butyl
- Butenyl
- Allyl
- Methylthiopropyl

SULFIDES
- Hydrogen
- Carbonyl
- Dimethyl
- Diethyl
- Dibutyl

DISULFIDES
- Carbon
- Dimethyl
- Methyl ethyl
- Diethyl
- Ethyl propyl
- Dipropyl
- Propyl butyl
- Propyl allyl
- Diallyl

TRISULFIDES
- Dimethyl

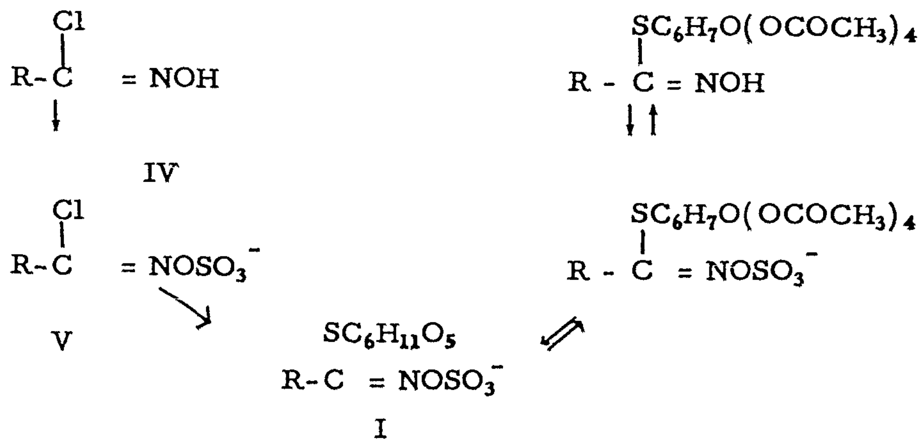
SLIDE 3. VOLATILE SULFUR COMPONENTS OF FRESH CABBAGE

$$(1) \quad \underset{\text{I}}{R-\overset{\overset{\displaystyle S\,C_6H_{11}O_5}{|}}{C}=NOSO_3^-} + H_2O \xrightarrow[\text{L-Ascorbate}]{\text{Enzyme}} \beta\text{-D-Glucose} + \underset{\text{II}}{R-\overset{\overset{\displaystyle S^-}{|}}{C}=NOSO_3^-} + H^+$$

$$(2) \quad \text{II} \rightarrow RNCS + SO_4^{=}$$

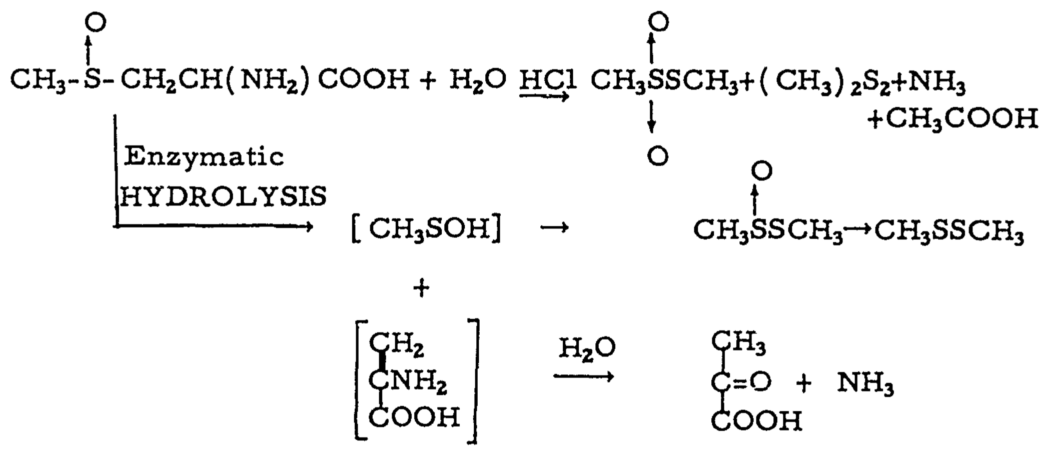
$$(3) \quad \text{II} + H^+ \rightleftharpoons [\, R-\overset{\overset{\displaystyle SH}{|}}{C}=NOSO_3^- \rightleftharpoons \underset{\text{III}}{R-\overset{\overset{\displaystyle S}{\|}}{C}-NHOSO_3^-} \,] \rightarrow RCN + S + HSO_4^-$$

SLIDE 4. HYDROLYSIS OF THIOGLUCOSIDES BY ENZYMES



R = Alkyl or Aryl

SLIDE 5. SYNTHESIS OF MUSTARD OIL GLUCOSIDES



SLIDE 6. ENZYMATIC AND ACID HYDROLYSIS OF S-METHYL CYSTEINE SULFOXIDE

Celery	Carrots	Oranges
Cabbage	Onions	Strawberries
Mustard	Garlic	Pineapple
Parsley	Milk	Raspberries
Horseradish	Tomatoes	Radish
Spinach	Bananas	Watercress, etc.

Slide 7. Processed Foods Improved in Flavor

by Addition of Enzymes